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# An Anatomical Assessment of Branch Abscission and Branch-base Hydraulic Architecture in the Endangered *Wollemia nobilis*

G. E. BURROWS[1,*], P. F. MEAGHER[2] and R. D. HEADY[3]

[1]*Institute for Land, Water and Society, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia,* [2]*Mount Annan Botanic Garden, Mount Annan Drive, Mount Annan, NSW 2567, Australia and* [3]*School of Resources, Environment and Society, The Australian National University, Canberra, ACT 0200, Australia*

• *Background and Aims* The branch-base xylem structure of the endangered *Wollemia nobilis* was anatomically investigated. *Wollemia nobilis* is probably the only extant tree species that produces only first-order branches and where all branches are cleanly abscised. An investigation was carried out to see if these unusual features might influence branch-base xylem structure and water supply to the foliage.
• *Methods* The xylem was sectioned at various distances along the branch bases of 6-year-old saplings. Huber values and relative theoretical hydraulic conductivities were calculated for various regions of the branch base.
• *Key Results* The most proximal branch base featured a pronounced xylem constriction. The constriction had only 14–31 % (average 21 %) of the cross-sectional area and 20–42 % (average 28 %) of the theoretical hydraulic conductivity of the more distal branch xylem. *Wollemia nobilis* had extremely low Huber values for a conifer.
• *Conclusions* The branch-base xylem constriction would appear to facilitate branch abscission, while the associated Huber values show that *W. nobilis* supplies a relatively large leaf area through a relatively small diameter 'pipe'. It is tempting to suggest that the pronounced decline of *W. nobilis* in the Tertiary is related to its unusual branch-base structure but physiological studies of whole plant conductance are still needed.

**Key words:** Hydraulic architecture, xylem, tracheids, compression wood, branch abscission, cladoptosis, Huber value, Wollemi pine, *Wollemia nobilis*, *Agathis*, *Araucaria*, Araucariaceae.

## INTRODUCTION

*Wollemia nobilis* (Araucariaceae), the Wollemi pine as it is commonly known, is a relatively new species to science. It was discovered in a canyon about 150 km north-west of Sydney in 1994 (Woodford, 2000) and named in 1995 (Jones *et al.*, 1995). Fewer than 100 adult plants are known (DEC, 2005), although fossil pollen evidence indicates that the species, or one or more of its forerunners, were once abundant and widespread in Australia (Macphail *et al.*, 1995; Dettmann and Jarzen, 2000). Two other extant genera are currently recognized in the Araucariaceae, *Agathis* (approx. 21 species) and *Araucaria* (approx. 19 species) (Setoguchi *et al.*, 1998, Hill and Brodribb, 1999). *Wollemia nobilis* is generally considered to be more closely related to *Agathis* than *Araucaria* (Gilmore and Hill, 1997; Stefanović *et al.*, 1998), although it is more similar to *Araucaria* in a range of features (Chambers *et al.*, 1998).

A remarkable feature of *Wollemi nobilis* is its branches. The first-order branches are plagiotropic, unbranched, relatively short-lived (usually 5–11 years), terminated by male or female strobili, shed as single units with all leaves still attached, and have a well-developed basal abscission zone (Fig. 1) (Hill, 1997; Offord *et al.*, 1999). As noted there is usually only a single order of branching and this is probably unique in extant woody plants. Damaged branches will sometimes branch again (Offord *et al.*, 1999), while branches may grow again once a strobilus has fallen

(P. F. Meagher and C. A. Offord, unpubl. res.). The branch leaf axils have buds or meristems (Burrows, 1999) but they remain inhibited unless the branch apex is damaged. The shed branches form a distinctive litter on the forest floor (Hill, 1997) and this was one of the main features that led to the species' discovery (Woodford, 2000).

*Wollemia nobilis* may possibly be the only extant tree species that does not form permanent structural branches. The 'branching' in the canopy comes from orthotropic epicormic secondary leaders that partially reiterate the architecture of the young adult plant (Hill, 1997; Burrows *et al.*, 2003). Examination of the proximal end of abscised *W. nobilis* branches shows a smooth separation layer and a small xylem protrusion (see Fig. 6). *Wollemia nobilis* is probably the only extant tree species where all branches are cleanly abscised. Branch abscission occurs in other woody species, but usually consists of the shedding of small secondary branchlets (Millington and Chaney, 1973; Addicott, 1982, 1991; Rust and Roloff, 2004). Abscission of larger and/or primary branches has been noted in only a few genera, with *Agathis* providing some of the best examples (Barnard, 1926; van der Pijl, 1952; Licitis-Lindbergs, 1956; Wilson *et al.*, 1998). In both *Agathis australis* and *Agathis alba* a substantial cortical swelling occurs at the branch base and within this swelling the branch-base xylem is constricted (Barnard, 1926; van der Pijl, 1952; Licitis-Lindbergs, 1956). It appears that the xylem constriction facilitates branch abscission (Licitis-Lindbergs, 1956).

* For correspondence. E-mail gburrows@csu.edu.au

FIG. 1. Tops of *Wollemi nobilis* leaders bearing numerous unbranched branches. Note the segmented outline of the branches with each segment representing one year's growth. Note also that the branches droop with age, which is almost certainly due to the progressively greater branch weight with increasing branch age and the relatively small cross-sectional area of xylem within the branch. It may also be related to the relatively weak attachment between the branch and stem. (Photograph J. Plaza.)

As *W. nobilis* abscises all its branches and is closely related to *Agathis* (Gilmore and Hill, 1997; Stefanović *et al.*, 1998) we wondered if *W. nobilis* also had an unusual branch-base vasculature. Initially a single *ex situ W. nobilis* sapling was examined. While a pronounced swelling of the branch base was not present, removal of the cortical tissues revealed a remarkable constriction of the branch-base xylem. It appeared that the constriction could facilitate branch abscission. The constriction was so pronounced that we surmised that it might also restrict water supply to the foliage under some conditions. This suggested that the constriction could have been associated with the marked decline of the species through the Cenozoic, leading to its current restricted distribution and abundance. Consequently, a morphological and anatomical study of the branch base was undertaken to assess the potentially conflicting requirements of facilitating abscission while providing water to the foliage.

## MATERIALS AND METHODS

Both *in situ* and *ex situ* materials of *Wollemia nobilis* were examined, both morphologically and anatomically. Morphological comparison was made with two species of *Araucaria* and one species of *Agathis*.

### *Wollemia nobilis*

The leaves on the branches and the main stem form repetitive growth units (Jones *et al.*, 1995), with each unit representing 1 year's growth (Fig. 1). This facilitates determining the age of branches and plants and degree of vigour. All leaves on a branch stay alive until the branch dies and the branch is shed with the leaves still attached. Branches with relatively intact leaves may remain on the forest floor for many years.

### Ex situ *plant sapling material*

Given the restriction on destructive sampling of the plants in the wild most research was performed on seed-derived cultivated plants growing in 75-L pots under 50 % shade-cloth at Mount Annan Botanic Garden (approx. 55 km SW of Sydney). The plants were 6–7 years of age, 2·0–2·5 m high and the main stem was 3–4 cm and 1·5–2·0 cm diameter at the base and tip, respectively.

### *Morphology, anatomy and scanning electron microscopy*

For anatomical studies, the vascular tissues of a branch base (similar to the extent of branch xylem shown in Fig. 3C but with the surrounding tissues intact) were excised from the main stem, and then cut into 4- to 5-mm-thick slices. Research concentrated on branches in the second and third growth units down from the main stem apex (i.e. about 20–90 cm down from the apex). These branches were 35–50 cm long, each with two or three growth units. Detailed anatomical measurements (see below) were made on four branches (two from each growth unit) from each of three plants, with further morphological and anatomical observations made on approx. 70 other branches from these three plants and an additional seven plants. Materials were fixed in 50 % formalin–acetic acid–alcohol, dehydrated through a graded ethanol series and infiltrated with Leica HistoResin® under a slight vacuum for a minimum of 2 d. The samples were placed in pharmaceutical gelatin capsules containing HistoResin® and polymerized overnight at 60 °C. They were then transverse sectioned at 4 μm using tungsten carbide-tipped steel blades fitted to a motorized retraction microtome. Sections were stained with 0·5 % toluidine blue and observed under bright field microscopy.

Digital photomicrographs were taken of sections of the constriction, region of maximum stranding and 1–2 cm distal to where the strands rejoined (the apparent branch base) (Fig. 3). These regions are shown in Fig. 3A and C as 'c' (constriction), 's' (strands) and 'abb' (apparent branch base). These images were then analysed with image analysis software (Image Pro Plus). Total cross-sectional area of xylem was measured. The apparent branch base was divided into upper normal/opposite wood and lower compression wood areas. Various anatomical measurements were then made on six evenly distributed sectors from around the xylem cylinder for the constriction and the strands or within the normal/opposite and

compression wood areas of the apparent branch base. The area of the sector was measured, the area occupied by ray parenchyma was measured, the number of tracheids was counted (usually between 200–400 tracheids per sector) and the lumen diameter of each tracheid measured. The lumens were rarely truly circular but the diameter was calculated based on the average of diameter measurements made at 2° intervals for each tracheid. As xylem cylinders were of small diameter (<1·0 cm) and no lumen occlusions were observed in sectioned or in freshly cut xylem it was assumed that all tracheids were functional. The tracheid lumen diameters were then grouped into 1-μm bands (e.g. 6–6·99 μm, 7–7·99 μm, etc.) and the theoretical hydraulic conductivity of the sector was calculated using the Hagen–Poiseuille equation (flow rate is proportional to the fourth power of the radius of the capillary) (Tyree and Zimmermann, 2002). Theoretical specific hydraulic conductivity was calculated, then the relative theoretical hydraulic conductivity of the constriction, strands and apparent branch base was calculated.

Several additional branch bases were excised from positions adjacent to those selected for anatomical examination, then cut into approx. 2-mm-thick transverse slices for scanning electron microscope (SEM) examination. After cutting, specimens were cleaned of cytoplasmic debris by washing in several changes of distilled water, dried at atmospheric pressure over silica gel for 48 h and under vacuum ($10^{-4}$ Pa) for 24 h, attached to mounting stubs and sputter coated with a 10-nm layer of gold. A Cambridge Instruments S360 SEM fitted with a high-brightness lanthanum hexaboride ($LaB_6$) electron source was used to image the various wood anatomical features.

### Huber values

The leaf areas of the branches selected for anatomical investigation were measured with a Licor LI-3100 area meter (all leaves were detached from the branch axis and individually measured) and using the measured xylem cross-sectional areas, Huber values [xylem cross-sectional area divided by the distal leaf area ($m^2 \, m^{-2}$)] were calculated at various planes along the branch base. Leaf dry weight was also measured so Huber values could also be expressed on a $mm^2 \, g^{-1}$ leaf weight basis for comparison with literature values expressed in this manner.

### In situ *adult material*

Two types of material were collected from adult trees in the Wollemi National Park: branches and main stem material.

*(1) Branch material.* Twenty fallen dead branches, mainly larger upper canopy branches, similar to those shown in Fig. 1, were collected from below several trees. In addition, ten smaller branches were collected from a fallen and partially decomposed trunk. The length of the branches, the number of annual growth units and leaf area were measured. The xylem projecting from the abscission zone was cut in transverse section (TS) at various distances from the tip,

digitally photographed under a dissecting microscope and xylem TS area measured and Huber values calculated.

*(2) Main stem material.* Two types of orthotropic main stem material and associated branch-base xylem were examined. The first type of main stem examined was completely dead and had been collected from the canyon floor. The stem was 220 mm in length, had about 20 branch abscission scars still apparent on the bark, the secondary xylem was 39–45 mm in diameter, and had 11 growth rings. Starting at an abscission scar the outer bark was scraped away to reveal the branch xylem that was located within the bark (Fig. 7) and the length and width of the xylem measured for 14 branch bases. For the fallen tree mentioned above several lengths of orthotropic stem were collected. The bark/cortex was highly degraded and the branches had mostly fallen away but some of the branch-base xylem constrictions remained intact and their cross-sectional dimensions were measured.

### Agathis robusta *(Queensland kauri pine),* Araucaria bidwillii *(Bunya pine) and* Araucaria cunninghamii *(Hoop pine)*

These species were chosen to make morphological comparisons with *W. nobilis* as they provide a wide taxonomic sample of the Araucariaceae and are extant in Australia. Plants of all three species were grown from seed. They were 2·5–3 m tall and growing in 75-L pots. For each species first-order branches from the fourth and fifth (for *Araucaria cunninghamii* and *Araucaria bidwillii*) or second and third (for *Agathis robusta*) pseudowhorls of branches back from the shoot apex were studied from each of two plants. The pseudowhorls of branches are further apart in *Agathis robusta* and thus the branches selected were functionally similar to those selected in the other species studied. The bark/cortex was excised and the form of the branch-base xylem was observed.

### Statistical analysis

Analysis of variance was used to statistically test for whorl (upper, lower), zone (constriction, strands, apparent branch base — opposite/normal, compression), whorl by zone effects, with individual trees as replicates. For each of the variables analysed (percentage ray parenchyma in TS, average tracheid lumen diameter, hypothetical specific hydraulic conductivity) least significant differences were calculated at the $P < 0.05$ level.

## RESULTS

### Morphology

First-order branches of *Araucaria bidwillii* and *Araucaria cunninghamii* are long-lived and are not abscised. First-order branches on the sampled plants all exhibited second-order and in some cases third-order branching. In both species the first-order branches met the main stem 10–30° above the perpendicular, with a slight dilation where a branch joined the stem (Fig. 2A, C). Removal of the bark and cortical tissues revealed that the secondary

xylem of the branches was essentially the same diameter where it exited from the main stem xylem compared with several centimetres further along the branch (Fig. 2B, D). The branch-base xylem was usually 5–7 mm in diameter with a pith 1–2 mm in diameter.

First-order branches of *Agathis robusta* saplings are cleanly abscised. The branches were at a similar angle to the perpendicular to that recorded for *Araucaria bidwillii* and *Araucaria cunninghamii*. The primary branch base of *Agathis robusta* had a pronounced swelling, particularly on the lower side (Fig. 2E) and it was not obvious where abscission would occur. Removal of the cortical tissues revealed that where it exited the main stem the xylem of the branch base had a narrow diameter (Table 1) and was
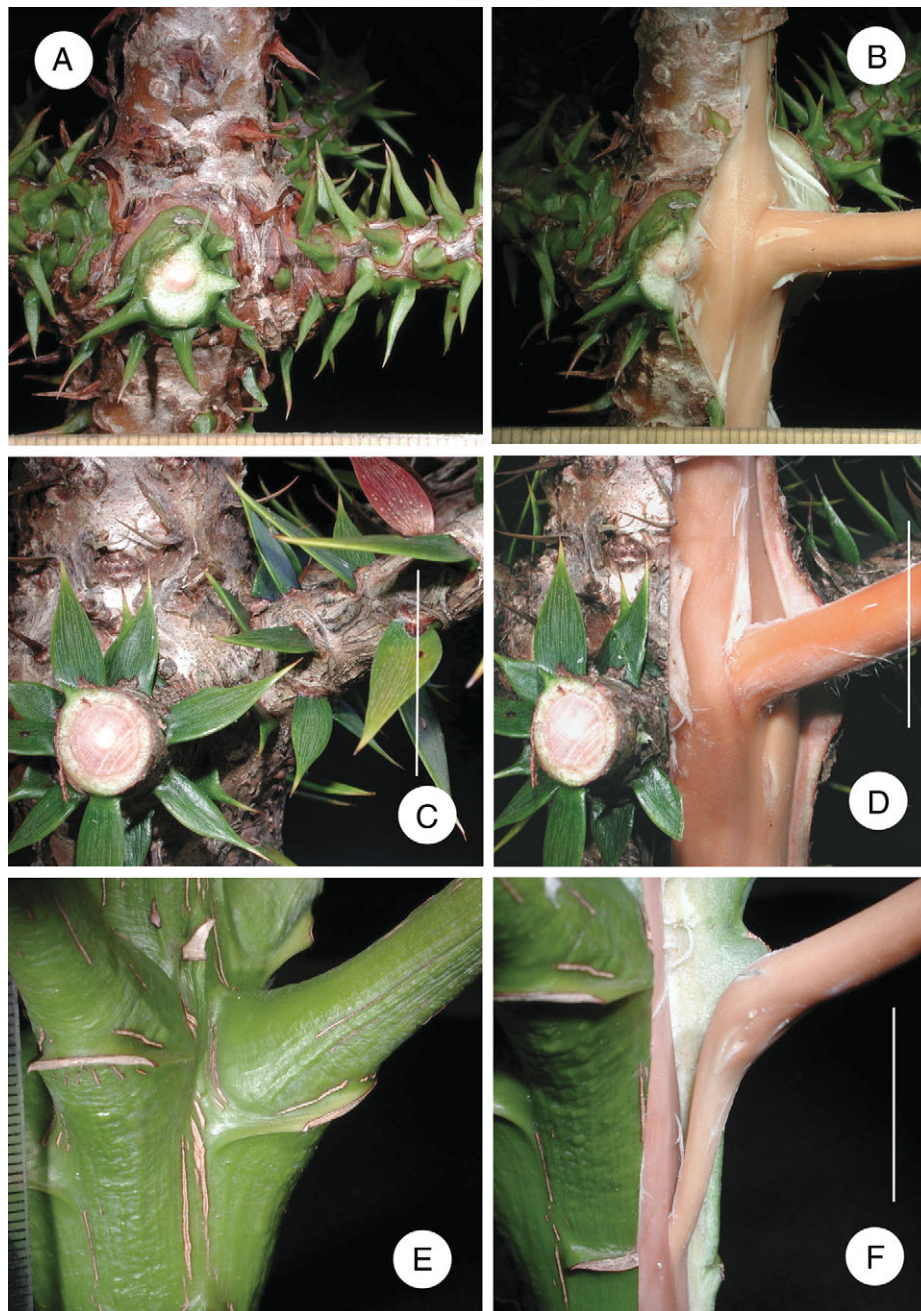


FIG. 2. Attachment of first-order branches to the main stem in saplings of three species of the Araucariaceae. In (A), (B) and (E) the scale is divided into millimetres while in (C), (D) and (F) the scale bar is 2 cm. (A) *Araucaria cunninghamii* showing a pseudowhorl of four branches. (B) As per (A) but with partial cortex/bark removal showing the branch-base xylem attaches directly to the main stem. Note that the branch-base xylem diameter is relatively uniform. (C, D) As per (A) and (B) but for *Araucaria bidwillii*. (E) *Agathis robusta* showing a pronounced swelling on the lower side of the branch attachment. (F) As per (E) but with the cortex partially removed showing that the branch-base xylem narrows and travels downward within the cortex before joining the main stem.

TABLE 1. *Various measurements of branch bases of saplings of* Agathis robusta *and* Wollemia nobilis

| Species and measurement | Constriction | Abscission area | Apparent branch base |
|---|---|---|---|
| *Agathis robusta* | | | |
|    Xylem radial extent (mm) | 2·0–2·9 | 5·0–6·4 | 5·0–6·7 |
|    Xylem tangential extent (mm) | 2·7–3·5 | 6·0–8·5 | 5·0–6·7 |
|    Pith diameter (mm) | 0·7–1·0 | 3·4–3·8 | 2·3–2·6 |
| *Wollemia nobilis* | | | |
|    Xylem radial extent (mm) | 0·9–1·5 | | 2·0–3·4 |
|    Xylem tangential extent (mm) | 1·0–2·1 | | 2·6–3·9 |
|    Pith diameter (mm) | 0·2–0·3 | | 0·5–2·1 |
| Huber value (average) | $4{\cdot}56 \times 10^{-5}$ (s.e. $\pm\, 2{\cdot}7 \times 10^{-6}$) | | $1{\cdot}95 \times 10^{-4}$ (s.e. $\pm\, 5{\cdot}3 \times 10^{-6}$) |
| Average percentage of ray parenchyma in TS | 20·8 (s.e. $\pm$ 1·01) | 25·0 (s.e. $\pm$ 1·04) | Opposite/normal 4·9 (s.e. $\pm$ 0·35) |
| | | | Compression 4·5 (s.e. $\pm$ 0·28) |
| Average tracheid lumen diameter ($\mu$m) | 11·1 (s.e. $\pm$ 0·10) | 9·8 (s.e. $\pm$ 0·22) | Opposite/normal 10·1 (s.e. $\pm$ 0·18) |
| | | | Compression 9·0 (s.e. $\pm$ 0·16) |

almost parallel to the main stem xylem for about 1 cm (Fig. 2F). It then increased in diameter (Table 1) and bent through approx. 45°, then narrowed slightly and had a relatively uniform diameter (Table 1) for several centimetres (Fig. 2F). *Agathis robusta* branches had a much larger pith than the two *Araucaria* species.

In *W. nobilis* saplings the branch base joined with the main stem without a pronounced swelling (Fig. 3B, D). In contrast to *Agathis robusta* each branch base sat in a 'socket' that projected slightly from the stem and a narrow groove encircled the base of the branch (Fig. 3B, D). This groove is the future site of branch abscission and was identifiable from an early age.

In saplings removal of the cortical and bark tissues revealed that in the first-formed branch bases (i.e. near soil level) there was a slight and short constriction in the branch xylem where it exited the main stem vascular tissues. In branches from about 1 m above soil level the branch-base xylem was similar to that described above for *Agathis robusta* except that rather than increasing in diameter at the bend (Fig. 2F) and developing a large parenchymatous pith, the xylem flattened out and divided into a number of separate or nearly separate strands (Fig. 3A).

In the upper branches the branch xylem exited the main stem xylem as a narrow diameter 'pipe' (Table 1; Fig. 3C, E). The xylem continued upwards as a single 'pipe' within the cortex for up to 6 cm (Fig. 3E) before it separated into several discrete or semi-discrete strands (usually between 0·3 and 0·8 mm in diameter) that then merged together in the almost cylindrical apparent branch base (Table 1; Fig. 3C, E). The strands were in a flattened plane and were bent through approx. 45°. SEM images (Fig. 4A–I), combined with a plot of xylem cross-sectional area (Fig. 5), revealed that the xylem divided into several strands before any substantial increase in xylem area occurred. At the distal end of the strands the xylem cross-sectional area increased rapidly in a short distance and where the strands rejoined was close to the maximum TS xylem area for a branch. This basic structure (narrow diameter xylem cylinder within the stem cortex, xylem dividing into strands in the abscission zone, and strands rejoining in

the much larger diameter apparent branch base) was observed in all upper branches in all saplings examined (ten plants, >100 branch bases dissected).

The base of abscised branches had an outer ring of periderm (the inner extension of the groove; Fig. 3B), a central area where the abscission zone had formed and jagged broken xylem strands on the upper edge (Fig. 6A). For larger branches the groove extended inwards for 2–3 mm (Fig. 6A) and thus the actual area of physical attachment was usually only about 60 % of the apparent area of attachment (Fig. 3B). On the basis of the complex of strands that remained in the bark/cortex after branch abscission (Fig. 7B) and the complex of strands that were found in the base of abscised branches (Fig. 6B) it appears that branch detachment usually occurs in the stranded region of the branch-base xylem. Longitudinal sections of the abscised branches showed a greater thickness of xylem on the lower side of the branch-base xylem and that the vascular tissues were located on the upper side of the branch base (Fig. 6C, D).

Based on the sapling plants it appeared that the later-formed branches had progressively longer lengths of constriction and the stranding became increasingly complex. These trends continued in the piece of large diameter main stem collected from the National Park. Scraping away the outer bark revealed that 4·0–6·5 cm of branch-base xylem remained within the bark/cortex, extending from the abscission scar to where the branch xylem entered the main stem xylem (Fig. 7A, B).

The branch xylem within the stem cortex (i.e. the constriction) was progressively enveloped within the secondary xylem of the main stem. This process pushes the most proximal branch xylem away from the centre of the main stem (Fig. 7C, D). Within the main stem wood there appeared to be excellent integration of the lower side branch xylem with the main stem xylem but the connection did not appear as well developed on the upper side (Fig. 7C). In stems with substantial secondary growth any external measurement of the length of the constricted region (e.g. Figs 3A, C, E and 7B) will underestimate its length compared with when it was first formed. For example, in the stem shown in Fig. 7B, 2 cm of
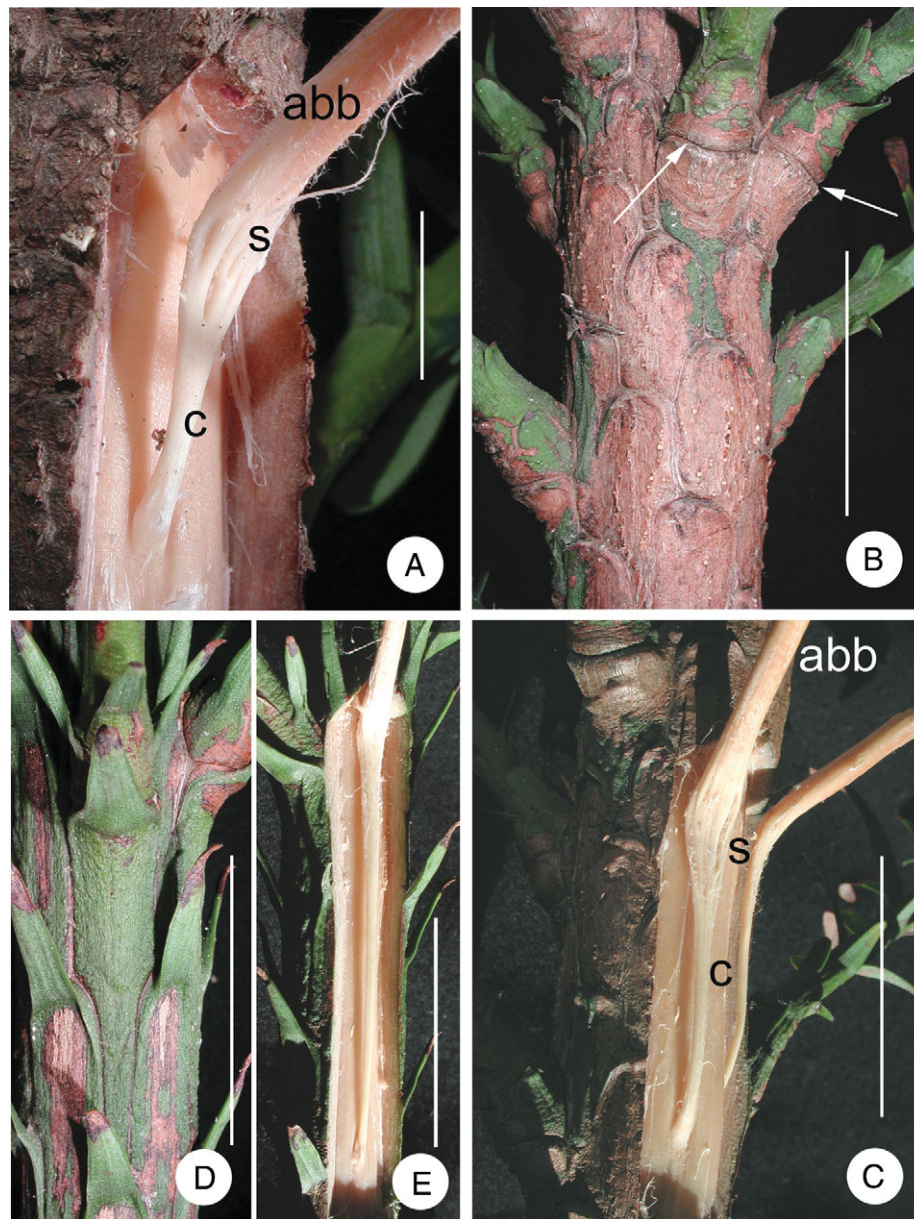
FIG. 3. First-order branch bases of 2–3 m high *Wollemia nobilis* saplings. (A) Branch base from about 1 m above soil level with partial cortex/bark removal. Note the relatively short and narrow diameter constriction (c) of the branch-base xylem, the partial stranding (s), and the much larger diameter of the apparent branch base (abb). Scale bar = 1 cm. (B–E) Branch bases from near the sapling apex. Scale bars = 3 cm. (B) Intact leader with several attached branches. Note the groove (arrowed) at the base of the branches. This is where the branches will abscise. (C) As per (B) but with partial cortex/bark removal showing the constriction, the flattened stranded region and the apparent branch base. (D, E) As per (B) and (C) but with about 6 cm of narrow-diameter branch-base xylem within the cortex.

branch-base xylem had been enveloped by the secondary xylem (Fig. 7C, D). Thus all branches on this piece of material had 6–8.5 cm of narrow-diameter xylem within the cortex when first formed. The constriction was between 1.0 and 1.3 mm in radial extent and 1.4 and 2.2 mm in tangential extent and the pith was 0.3–0.5 mm diameter (Fig. 7D), giving a cross-sectional area of 0.9–2.1 mm$^2$.

The abscised branches from the National Park were 70–120 cm (average 98 cm) long, had 6–13 growth units, a leaf area of 406–1648 cm$^2$ (average 902 cm$^2$), a total of 1.9–8.0 mm$^2$ (average 4.9 mm$^2$) of xylem in several strands at the proximal tip (Fig. 6A, B) and 6.7–27.8 mm$^2$ (average 17.4 mm$^2$) of xylem 2–3 cm distal to the proximal tip. Given that the cross-sectional area of the xylem in the proximal tip of the abscised branches was almost certainly greater than that of the constricted region that remained in the stem, it is probable that these branches had no more than 2–3 mm$^2$ of xylem in the constriction.
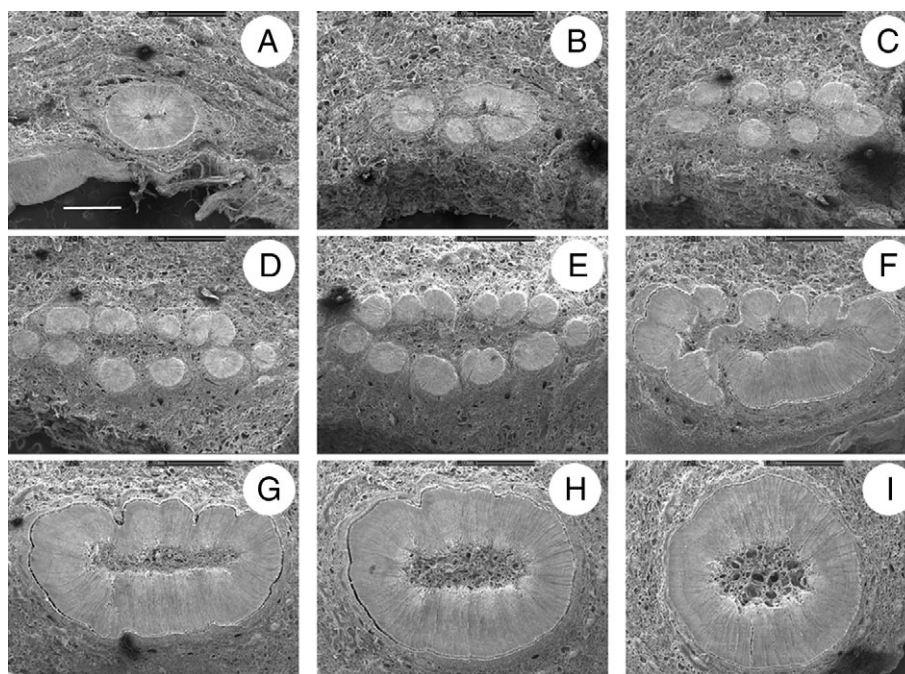
FIG. 4. SEM images of *Wollemia nobilis* branch-base xylem cut in transverse section. The branch base was very similar to those shown in Fig. 3C and was cut into approx. 2-mm slices over a distance of about 6 cm. All images are the same magnification. Scale bar = 1 mm. The slices were the following distances (mm) from where the constriction exited the main stem xylem: (A) 2, (B) 22, (C) 30, (D) 32, (E) 34, (F) 36, (G) 38, (H) 40 and (I) 48 mm.

*Huber values*

Huber values were calculated using the xylem TS area in the constriction (the *ex situ* saplings) and the xylem TS area just distal to where the strands rejoined in the apparent branch base (the *ex situ* saplings and the abscised branches from the National Park).

Average Huber values were 4·2 times greater in the apparent branch base than in the constriction (Table 1). Figure 8 shows a highly significant relationship between leaf area and xylem TS area for both the constriction ($n = 39$, $r^2 = 0.42$, $P < 0.001$) and the apparent branch base ($n = 52$, $r^2 = 0.94$, $P < 0.001$).
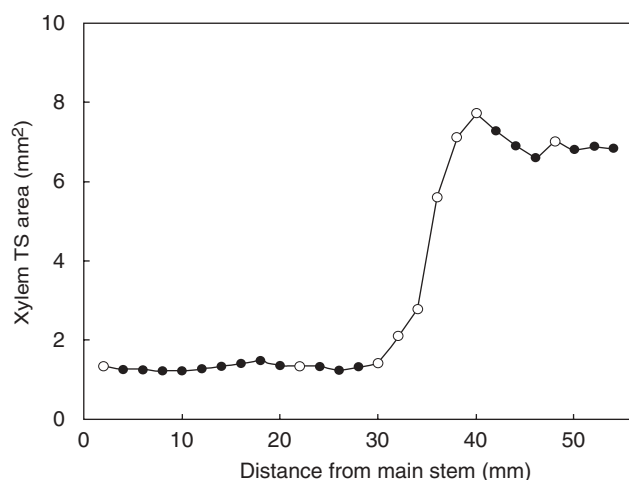


FIG. 5. Graph of the change in xylem cross-sectional area (mm²) in the branch base of a *Wollemia nobilis* sapling. The graph is based on the branch base shown in Fig. 4. Note that the constriction (0–20 mm) had a relatively consistent diameter and that some substantial division into strands occurred between 20 and 32 mm with little increase in xylem area. Note also the rapid increase in xylem area in the region just distal to the region of maximum stranding. The nine open circles correspond to the nine xylem sections shown in Fig. 4A–I.

*Anatomy*

The xylem in the constriction was elliptical in cross-section, with a small, central parenchymatous pith (Fig. 9A). A number of growth rings, equivalent to the number of foliage growth units, were usually apparent. The largest tracheid lumen diameters were usually found in the first-formed tracheids near the pith and the largest of the later-formed tracheids were smaller. Rays were prominent (Table 1) with individual parenchyma cells to 35 μm wide (Fig. 10A).

In the strands xylem structure was similar to that in the constriction, with an even higher percentage of ray parenchyma (Table 1) (Figs 9B and 10B). The vascular cambium of each strand either formed only to the outside of the strand, or formed a complete loop but produced far more xylem to the outside of the ring of strands than to the inside. In the latter, the small pith of the strand was located to the inside of the ring of strands (Fig. 9B). No obvious abscission zone was detected in the strands.

In the apparent branch base the xylem was usually wider on the lower side of the branch than the upper (Fig. 6D). Average ray parenchyma percentage in the opposite/ normal and compression wood were not significantly
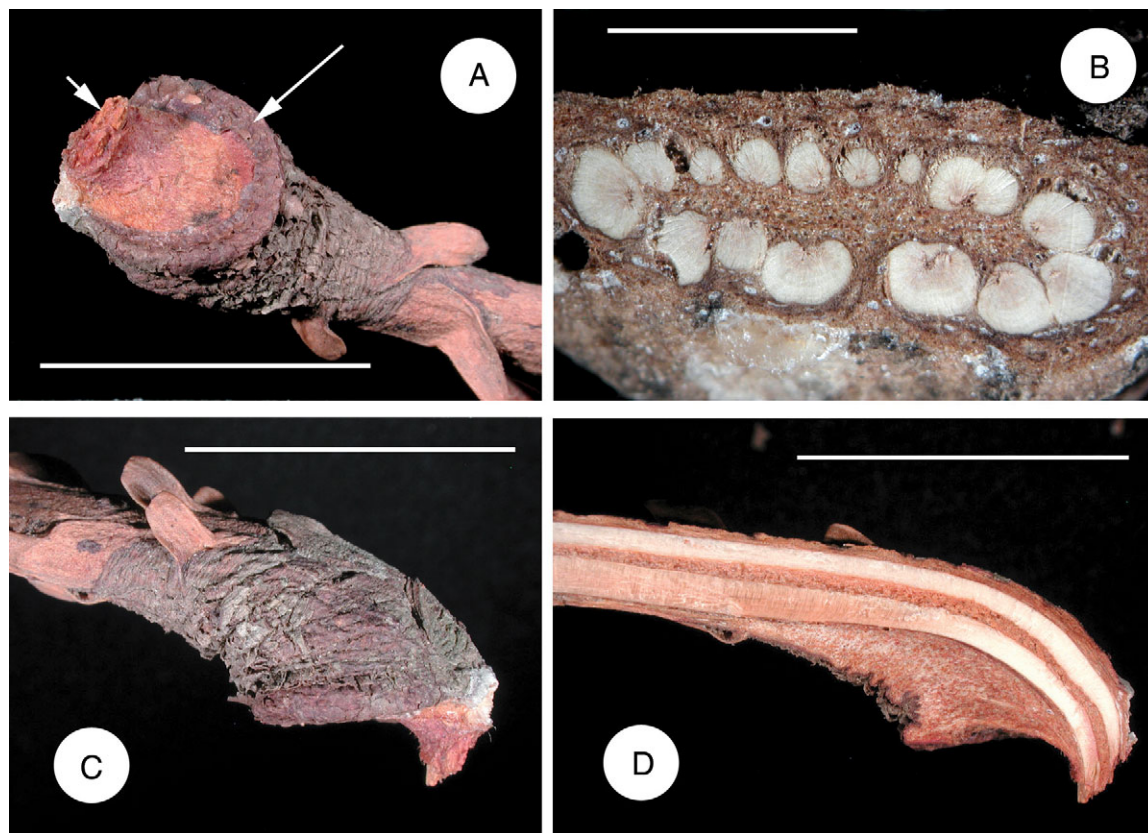
FIG. 6. Images of the bases of abscised *Wollemia nobilis* branches collected in the Wollemi National Park. (A) View of abscission zone showing that the external groove extended inward for 1–2 mm (long arrow), and that the lighter coloured area of attachment included the ridge of broken xylem (short arrow). Scale bar = 2 cm. (B) Detail of a xylem stub trimmed to reveal 16 separate or nearly separate xylem strands. Scale bar =5 mm. (C) Side-on view of a branch base. Scale bar = 2 cm. (D) As per (C) but cut in radial longitudinal section showing the greater depth of xylem on the lower side of the branch. Scale bar = 2 cm.

different but were significantly less than in the constriction and strands (Table 1). Within each growth ring the first-formed tracheids (earlywood) had relatively large lumens (Fig. 10D). Compression wood (rounded cells, thick walls, small lumens; Fig. 10C) was best developed in the latewood, particularly in the later-formed growth rings on the lower side of the branch (Fig. 9C). For the purposes of the theoretical hydraulic conductivity measurements, the compression wood was usually estimated to be about 40 % of the TS area (Fig. 9C). Note that within the compression wood sectors the earlywood tracheids had relatively large lumen diameters.

Average tracheid lumen diameter for the constriction, strands and apparent branch base (normal/opposite and compression wood) were 11·1, 9·8, 10·1 and 9·0 μm, respectively, and were all significantly different (Table 1). Examination of the frequency distribution of the tracheid lumen diameter ranges shows that the constriction and the opposite/normal wood of the apparent branch base had a relatively similar profile and a relatively even spread of diameters (Fig. 11). In contrast, the strands and the compression wood of the apparent branch base had a higher proportion of narrower tracheids (Fig. 11). Although fewer in number the larger diameter tracheids made a greater contribution to theoretical flow (Fig. 12).

*Relative hypothetical hydraulic conductivity*

The theoretical specific hydraulic conductivities of the four regions sampled were all significantly different. As expected the theoretical specific hydraulic conductivity of the apparent branch base compression wood was the lowest of the four areas (Fig. 10) sampled. Compared with the compression wood the theoretical specific hydraulic conductivity of the opposite/normal wood and the wood of the constriction were, on average, 1·7 (s.e. ± 0·09) times and 1·9 (s.e. ± 0·11) times greater, respectively. However the cross-sectional area of the apparent branch base was, on average, 5·1 (s.e. ± 0·3) times greater than the constriction (e.g. Figs 4, 5 and 9) and the area of normal/opposite wood in the apparent branch base was greater than the amount of compression wood. The theoretical hydraulic conductivity of the branch base was, on average, 3·7 (s.e. ± 0·2) times greater than that of the constriction.

## DISCUSSION

As noted, *W. nobilis* has apparently unique features for an extant tree species — all branches are unbranched and all branches are cleanly abscised. Apparently associated with facilitating branch abscission is a highly modified
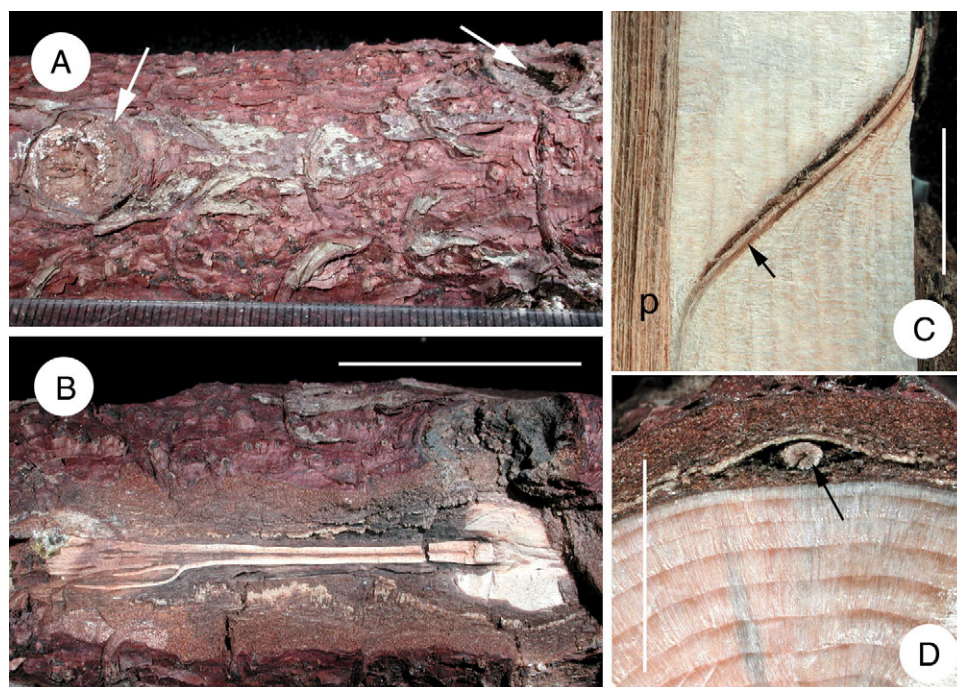
Fɪɢ. 7. Images of a length of fallen *Wollemia nobilis* main stem, approx. 42 mm diameter, collected in the Wollemi National Park. (A) External view showing two branch abscission zones (arrowed) and the extensive bark formation. Note that the former positions of the leaves are still apparent on the bark. Scale in millimetres. (B) Similar to (A) but with some bark removed revealing the branch-base xylem running for about 6 cm within the cortex. Note the extensive stranding near the abscission zone. Scale bar = 3 cm. (C) Radial longitudinal view of stem showing how the constriction is gradually incorporated into the stem secondary xylem with increase in stem diameter. Note that the constriction xylem appears well integrated into the stem on its lower side but not the upper. p, Stem pith. The arrow indicates pith of the constriction. Scale bar = 1 cm. (D) TS of stem showing the constriction (arrowed, about 1·3 mm deep × 2·0 mm wide) within the cortex. The lighter coloured band to the outside of the constriction is probably sclereids. Scale bar = 1 cm.

branch-base xylem structure that features a pronounced constriction that may travel for several centimetres within the stem cortex before separating into multiple strands in the abscission region. This structure represents an extreme modification of the first-order branch-base structure known from a small number of other species in the Araucariaceae.

*Xylem comparisons*

Heady *et al.* (2002) provided detailed measurements of *W. nobilis* wood. Earlywood tracheids in mature wood are larger than in juvenile wood (average: 40·1 µm *vs.* 26·9 µm radial, 31·1 µm *vs.* 18·3 µm tangential). Average lumen diameters were given for the earlywood (32·4 µm) and latewood (11·0 µm) but only for mature wood and not for saplings. Lumen diameters in the branch base of saplings in the present study were between 4 and 22 µm (Fig. 11). Average lumen diameters of tracheids from leaves of mature morphology were 5·5 µm and the largest lumens are <10·5 µm (Burrows and Bullock, 1999; G. E. Burrows, unpubl. res.). Thus *W. nobilis* shows the usual reduction in average tracheid lumen diameter from main stem to branch to leaf. Heady *et al.* (2002) recorded that ray volume for *W. nobilis* stem wood was between 6·3 and 8·3 %, which is within the usual range of 6–10 % for gymnosperms (Gartner, 1995). The average ray volume (4·7 %) in the apparent branch base was slightly

lower than the normal range, while average ray volume in the constriction (20·8 %) and the strands (25·0 %) was remarkably high and indicates that this wood may be mechanically weak.
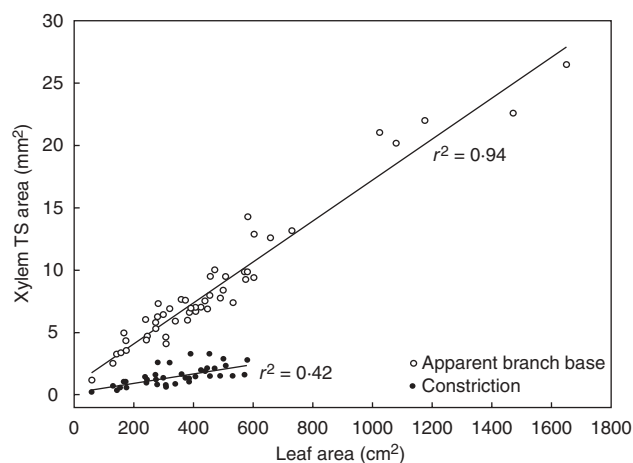


Fɪɢ. 8. Graph showing the increase in branch xylem cross-sectional area (mm$^2$) with increasing leaf area per branch (cm$^2$) for the constriction and the apparent branch base for *Wollemia nobilis*. For most branches, the xylem TS area of both the constriction and apparent branch base were assessed but most of the largest branches were collected as fallen branches and thus the constriction could not be assessed.
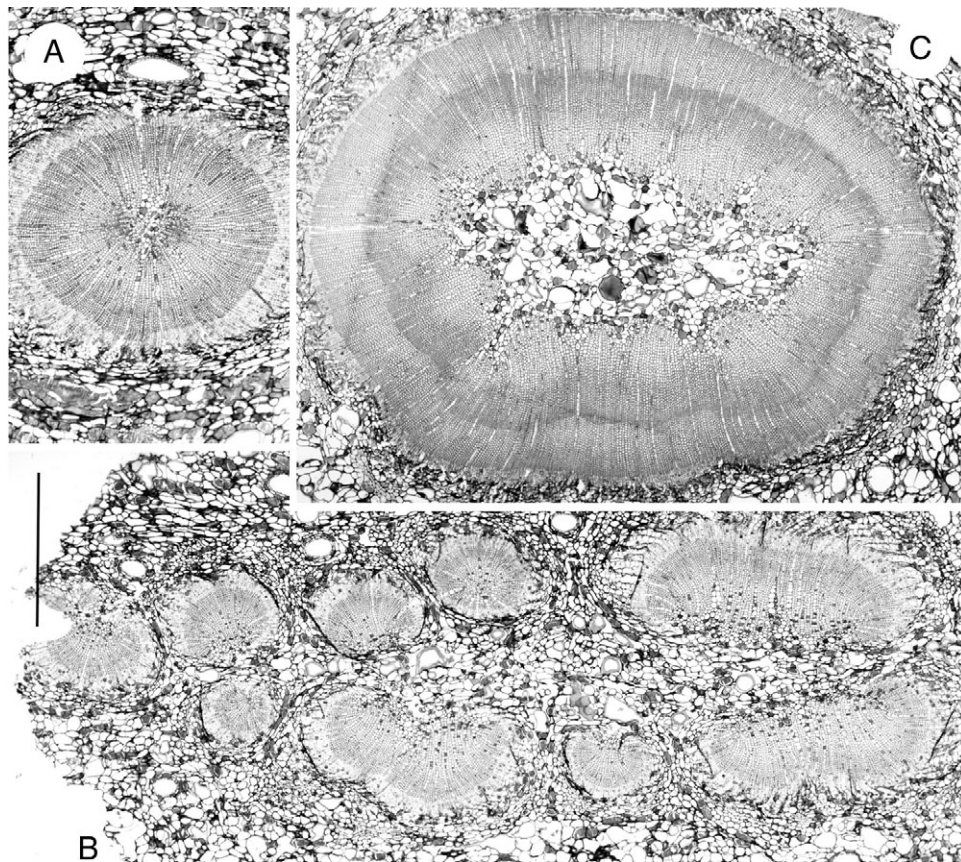
Fɪɢ. 9. Transverse sections of the xylem from a single *Wollemia nobilis* sapling branch base. All images are at the same magnification. Scale bar = 1 mm. (A) Constriction. (B) Region of maximum stranding showing about ten discrete or nearly discrete strands and that the strands are flattened into a narrow elliptic shape. (C) Apparent branch base showing two growth rings, with their earlywood and latewood. Note the greater development of compression wood (darker shade) on the lower side of the branch. Note also that the branch xylem in (C) is 4·7 times the area of that in (A). Xylem TS areas in (A–C) are 1·6, 2·7 and 7·5 mm$^2$, respectively.

### Huber values

In *W. nobilis*, Huber values based on the xylem TS area in the apparent branch base ranged between 1·25 and $3·23 \times 10^{-4}$, with an average of $1·95 \times 10^{-4}$. Given the wide diversity of material examined (branches from saplings and abscised branches from mature trees and a range in leaf area from 59 to 1648 cm$^2$) these Huber values of the apparent branch base were remarkably consistent (Fig. 8). Huber values based on the constriction were, on average, 4·24 times lower (range $2·2–9·4 \times 10^{-5}$, average $4·6 \times 10^{-5}$) than those recorded for the apparent branch base. Recently, numerous Huber and leaf : wood area ratios ($A_L/A_S$) values for tropical, Mediterranean and temperate gymnosperms have been published. Analysis of 15 studies from 1996 onwards covering 36 species in 16 genera (data available from the authors) gave an average Huber value of $5·0 \times 10^{-4}$ ($n = 55$, s.e. $\pm 0·39 \times 10^{-4}$). Most of these studies provided a range of Huber values for a single species and the average value given above was calculated using the lowest value provided in a study. The lowest value in these studies was about $1·2 \times 10^{-4}$ for *Pseudotsuga menziesii* (Spicer and Gartner, 1998*a*) and *Abies lasiocarpa* (DeLucia *et al.*, 2000). In a detailed

study Burgess *et al.* (2006) provided a range of Huber values for branches of *Sequoia sempervirens* ranging between 1·2 and $5·0 \times 10^{-4}$. Some caution is needed when making comparison with these values, e.g. stems of different diameters were measured, leaders and branches were measured and the xylem area was measured in different ways (sapwood only/all wood/wood plus pith, etc.). Nevertheless, it would appear that, based on the xylem TS area in the apparent branch base, *W. nobilis* has Huber values in the low to mid-region of the usual range for gymnosperms. Based on the constriction, *W. nobilis* had some of the lowest Huber values ever recorded for a gymnosperm. The average constriction-based Huber value for *W. nobilis* is about ten times lower than the literature-based average given above and about three times lower than the lowest literature-based value. The constriction had an unusually high percentage (average 20·8 %) of ray parenchyma. If this parenchyma were not present the Huber value would be even lower. In summary, it appears that *W. nobilis* supplies a relatively large leaf area through a relatively small diameter 'pipe'.

The hidden nature of the constriction in *W. nobilis* means that to simply cut off a branch a few centimetres from the
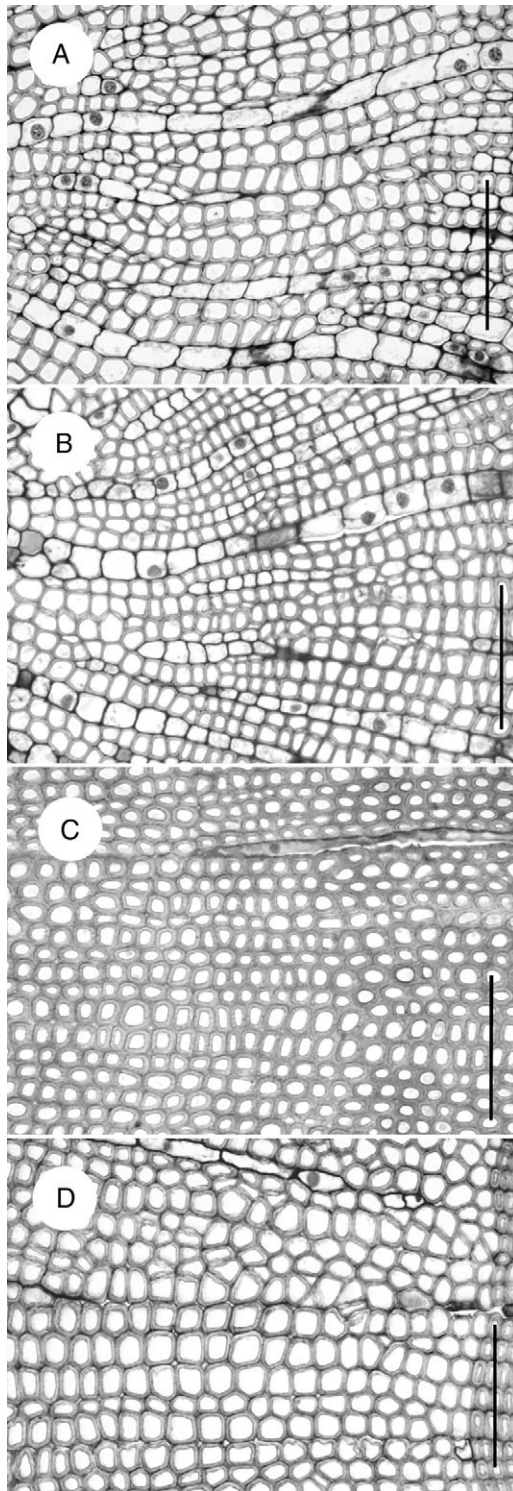
FIG. 10. Transverse sections of the xylem from *Wollemia nobilis* branch bases showing cell detail. All images are at the same magnification. Scale bar = 100 μm. (A) Constriction. Note the relative abundance of ray parenchyma cells. (B) Strands. (C) Apparent branch base, compression wood. (D) Apparent branch base, normal/opposite wood. Ray parenchyma percentage of cross-sectional area shown in (A–D) is 25, 26, 2 and 2 %, respectively. Average tracheid lumen diameter (μm) in (A), (B), (C) and (D) is 10·9, 9·5, 8·0 and 11·4, respectively. Theoretical specific conductivities of (A), (B) and (D) are 2·9, 2·5 and 3·3 times greater than (C), respectively.
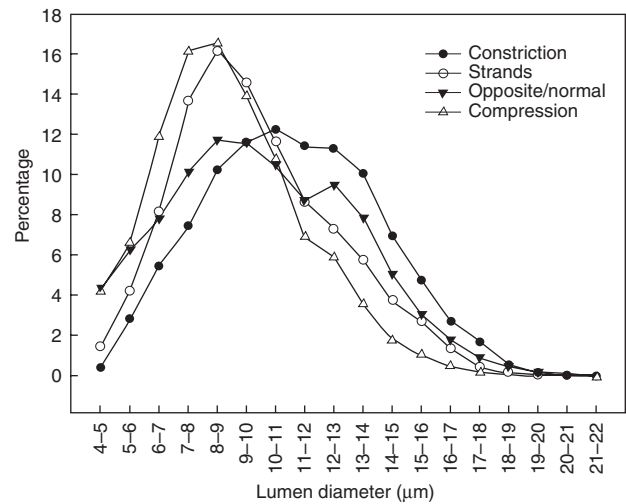


FIG. 11. Distribution of tracheid lumen diameters for the constriction, strands and apparent branch base (compression and opposite/normal wood) in the branch base of *Wollemia nobilis* saplings.

stem would result in potentially misleading results in hydraulic studies and in Huber value calculations. It is possible that a similar situation may have occurred in studies of *Agathis lanceolata* (Brodribb and Feild, 2000).

### Comparative lumen diameter

Based on Huber values it appears that *W. nobilis* supplies water to a relatively large leaf area through a relatively narrow 'pipe'. However, the constriction may have tracheids with unusually large lumen diameters and/or a frequency distribution biased toward large-diameter
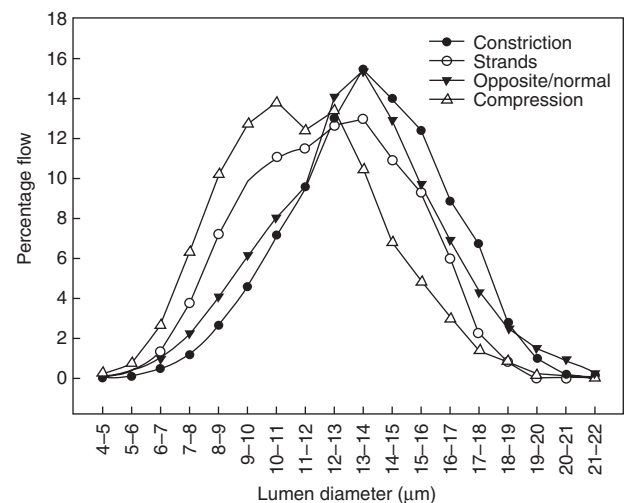


FIG. 12. Percentage contribution of various tracheid lumen diameter size classes to theoretical flow for the constriction, strands and apparent branch base (compression and opposite/normal wood) in the branch base of *Wollemia nobilis* saplings.

tracheids. Few studies are available that provide a break-down of frequency of tracheid lumen diameter in conifer branches (e.g. Spicer and Gartner, 1998*b*; Froux *et al.*, 2002; Mayr and Cochard, 2003; Mayr *et al.*, 2003; Ladjal *et al.*, 2005). Although statistical analysis is not possible it would appear that the tracheids of the *W. nobilis* branch constriction (Fig. 11) are probably of larger lumen diameter than those measured by Mayr and Cochard (2003) and Mayr *et al.* (2003) for *Picea abies*, but are smaller or similar to those of the seven species investigated by Spicer and Gartner (1998*b*), Froux *et al.* (2002) and Ladjal *et al.* (2005). Although far from conclusive it appears that the tracheid lumens of the *W. nobilis* branch constriction are not unusually large and probably would not provide a specific hydraulic conductivity remarkably higher than in other gymnosperm branches.

### Relative theoretical hydraulic conductivity

The principal reason for assessing theoretical hydraulic conductivity was to determine how the theoretical conductivity of the constriction compared with that of the apparent branch base, i.e. was the constriction just a morphological feature or was it also possibly a hydraulic constriction. An interesting component of this assessment was that the constriction was apparently fully supported within the main stem cortex and would probably have little or no mechanical support function, as indicated by the high parenchyma content and the absence of compression wood (i.e. a mechanical parasite; Tyree and Ewers, 1991). Consequently it would be reasonable for tracheid diameter in the constriction to be considerably larger than in the apparent branch base.

The constriction had a higher proportion of tracheids with larger lumen diameters than the strands or the apparent branch base (Fig. 11) and also had a significantly greater theoretical specific conductivity than either the compression wood or the normal/opposite wood of the apparent branch base. While the theoretical specific conductivity of the constriction was significantly greater than that of the apparent branch base the difference was not substantial. For example, the theoretical specific conductivity of the constriction was, on average, only 11 % greater than that of the normal/opposite wood. This, combined with the small cross-sectional area of the constriction, meant that the constriction, on average, had only 28 % of the relative theoretical conductivity of the apparent branch base. In summary, the study shows that the constriction is both a morphological constriction and a theoretical hydraulic constriction.

### Other possible restrictions to water flow in W. nobilis

Apart from the branch-base xylem constriction other possible restrictions to water flow may exist in *W. nobilis*. As noted, *W. nobilis* forms epicormic orthotropic secondary leaders (Hill, 1997) so that new branches can form in the canopy. These new leaders form from orthotropic axillary meristems in the leaf axils of the leader (Burrows *et al.*, 2003). In their dormant or near-dormant state these meristems or bud primordia have little or no associated vascular development or, at most, form a 'closed loop' of vascular tissues in the cortex immediately behind the bud primordium (Burrows *et al.*, 2003). In most plants axillary branch buds develop close to the apical dome and their vascular system is well integrated with the vascular tissues of the parent axis, but in *W. nobilis* if a dormant orthotropic meristem or bud commences active development it forms a vascular connection to the xylem and phloem of the stem by dedifferentiation of the cortical parenchyma (G. E. Burrows, unpubl. res.). Due to the presence of individual sclereids and sclereid bands (Fig. 7D) the vascular connection may take a very indirect path (Burrows, 1989) and must then integrate with the existing secondary xylem and phloem. It could be that these connections are both mechanically and hydraulically inefficient. This is somewhat similar to the study of Spicer and Gartner (1998*a*) who investigated branches of *Pseudotsuga menziesii* that had replaced leaders (branch-leaders). Fifteen months after leader removal, branch-leaders were still intermediate between branches and leaders in their water supply capacity. In addition most plants of *W. nobilis* have a self-coppicing habit and form numerous basal orthotropic stems from a common base (Hill, 1997; Offord *et al.*, 1999; DEC, 2005). This form of sprouting is rare in gymnosperms and the root and shoot systems may not be as well integrated as in a plant with a single stem.

Brodribb and Hill (1997) noted that most southern conifers possess waxy stomatal plugs, as does *W. nobilis* (Chambers *et al.*, 1998). In species with exposed stomata and wax plugs, maximum leaf conductance was only about 35 % of that which would occur if the plugs were absent (Brodribb and Hill, 1997). While this could be interpreted as a feature designed to restrict water loss in xeric environments, Brodribb and Hill argue that it is probably a feature associated with keeping the stomatal antechamber free of water in wet conditions. By slowing evapotranspiration the plugs may reduce the impact of any flow reduction related to the xylem constriction at the branch base.

*Wollemia nobilis* leaves are long-lived. As branches (Fig. 1) may live for 5–15 years before abscission it follows that the proximal leaves on a branch would also be alive for this length of time. Lusk *et al.* (2003) gives leaf longevities of about 2–7 years for 19 conifer species. Thus for the constriction to be at maximum conductivity all tracheids would need to remain functional for up to 15 years. If tracheids in the constriction are blocked and the first-formed tracheids are blocked first this would have a large impact on conductivity as these tracheids have the largest diameters.

### Hydraulic bottlenecks

Main stem to branch and branch to branch junctions are known to be hydraulic bottlenecks in both gymnosperms and angiosperms (Zimmermann, 1978; Ikeda and Suzaki, 1984; Tyree and Alexander, 1993; Lo Gullo *et al.*, 1995; Klugmann and Roloff, 1999; Rust and Roloff, 2002; Rust *et al.*, 2004). In several of the species investigated, the flow constriction was not due to a local drop in Huber

value, but was associated with smaller-diameter conducting cells, fewer vessels per square centimetre, and/or a high frequency of vessel ends (Ikeda and Suzaki, 1984; Lo Gullo *et al.*, 1995; Tyree and Zimmermann, 2002; Rust *et al.*, 2004). In contrast, in *W. nobilis* the lower theoretical conductivity was mainly related to cross-sectional area of xylem (Figs 3, 4 and 9) as average tracheid lumen diameter was greater in the constriction than in the more distal regions (Figs 10 and 11). Tyree and Ewers (1991) suggested that 'a species with severe hydraulic constrictions can support fewer orders of branches than those with mild or non-existent constrictions'. With subsequent physiological measurements, *W. nobilis* might become an excellent example to support their hypothesis.

In some species these flow constrictions are considered to be a relatively minor part of whole tree hydraulic performance (Tyree and Ewers, 1991; Tyree and Zimmermann, 2002). Tyree and Zimmermann (2002) concluded that 'branch junctions are an interesting anatomical anomaly, but are unlikely to have much impact on the overall water relations of trees'. Similarly Becker *et al.* (1999) found that the branches of conifers are less conductive than those of angiosperms but whole-plant conductances were similar and Spicer and Gartner (2002) found that severely bent Douglas fir seedlings had a severe reduction in specific conductivity due to compression wood formation but this had little impact on leaf-level processes. In some other species (e.g. branch abscission species) branch-base bottle-necks are ascribed a greater importance (Rust *et al.*, 2004).

### Evolutionary/palaeobotanical considerations

Australia's separation from Antarctica began approx. 97 Myr ago (Hill, 2004). In the Late Cretaceous, Australia was inside the Antarctic circle and the climate featured long dark winters, summer light was continuous but low in the sky, $CO_2$ levels were more than ten times current levels (>4000 ppm) (Sperry, 2003; Hill, 2004), and rainfall was probably reasonably high and reliable all year round (Hill, 2004). During the Mid-Cenozoic the Australian climate became drier and more seasonal as the continent drifted north, with severe aridity and widespread conditions like the present setting in by approx. 2·5 Myr ago (Crisp *et al.*, 2004; Hill, 2004).

The earliest unequivocal Araucariaceae date from the Jurassic (Kershaw and Wagstaff, 2001). The Araucariaceae radiated in the Late Cretaceous and Early Cenozoic, but were depleted by extinctions from the Mid-Cenozoic onwards (Crisp *et al.*, 2004). Overall Araucariaceae species numbers in Australia have declined from 36 fossil species to six extant species (Crisp *et al.*, 2004). Conifers, from several families, were extremely common in the Oligocene in south-eastern Australia. Extinction of many of these was probably driven by declining water availability (Hill, 2004).

Macrofossils comparable to *W. nobilis* date back to the early Cretaceous (Kershaw and Wagstaff, 2001), with the first reliable occurrences of the distinctive *Dilwynites* pollen from the Late Cretaceous (Macphail *et al.*, 1995). *Dilwynites* pollen is most abundant in the Palaeocene to Middle Eocene and is occasionally the dominant element (Hill and Brodribb, 1999). It has been recorded from Tasmania to north of Perth, Western Australia (Macphail *et al.*, 1995). It declines in abundance in the Late Eocene (Hill and Brodribb, 1999) and the last pollen records are from about two million years ago (Macphail *et al.*, 1995).

It appears that branch abscission was present in an early ancestor of the Araucariaceae as *Agathis* and *Araucaria* feature abscission of second- and/or third-order branches and *Agathis* and *Wollemia* feature abscission of at least some first-order branches. Associated with branch abscission is some degree of branch-base xylem constriction. On the available evidence the constriction is not pronounced in *Agathis* or *Araucaria* (see next section). For reasons unknown the constriction in *W. nobilis* is extreme in terms of length, diameter and stranding (Figs 3 and 7). The stranding may assist the xylem to break. The high percentage of parenchyma may assist the xylem to break and may also assist in protecting (wound periderm or tyloses) the plant against pathogen attack. This may have been of importance in warm humid conditions. In contrast, it is difficult to suggest any benefit for a very narrow-diameter branch-base xylem that travels for several centimetres within the stem cortex (Figs 3E and 7B).

Low light levels, high $CO_2$ levels and mesic conditions were probably present during the evolution and period of maximum distribution and abundance of *Wollemia*. These conditions would have created minimal transpirational demand and thus the development of extreme forms of xylem constriction may not have been selected against. As drier conditions developed in Australia the constriction may have limited the time and degree of stomatal opening. This could reduce photosynthesis and thus reduce the competitive abilities of *W. nobilis* seedlings, saplings and trees. To place this speculation in context consider that several branches from the saplings and the adult trees had 500–600 $cm^2$ leaf area supplied by 1·5–2·0 $mm^2$ of xylem (i.e. a very low Huber value). Given that about 20 % of the xylem TS area is occupied by non-conducting parenchyma and tracheid lumen diameters are not unusually large, then it seems reasonable to suggest that, in comparative anatomical terms, *W. nobilis* foliage has a relatively poor vascular supply. If true this could limit photosynthesis, which could limit competitiveness, which in turn could have played a part in the species pronounced decline and its final refuge in a mesic canyon.

### Branch abscission

Branch shedding is well developed in the conifers and is also common in some angiosperm trees (Millington and Chaney, 1973; Addicott, 1982, 1991). It is also well represented in the fossil record (Thomas and Cleal, 1999). Branch abscission in many species is associated with the loss of small, relatively young leafy shoots as a mechanism for adjusting canopy structure and/or water balance under stressful conditions (Addicott, 1982; Rust *et al.*, 2004). In *W. nobilis*, given that branches usually grow for 5–11 years, branch abscission is probably not a technique for quickly reducing leaf area during times of stress.

Relatively few araucarian species abscise individual leaves and as such branch abscission could be considered a family characteristic. While xylem constrictions associated with branch abscission have apparently only been described previously in two *Agathis* species (van der Pijl, 1952; Licitis-Lindbergs, 1956) the present study adds a further two species (*W. nobilis* and *Agathis robusta*). Short and simple xylem constrictions have also been observed where the second-order branches join a first-order branch in three other *Araucaria* species (*A. bidwillii*, *A. cunninghamii* and *A. heterophylla*) (G. E. Burrows, unpubl. res.).

There have been only two relatively detailed studies of branch abscission in the Araucariaceae (Licitis-Lindbergs, 1956; Wilson *et al.*, 1998). Both studies were of *Agathis australis* (New Zealand kauri) and both studies were largely morphological investigations of the abscission process, with little or no consideration of the hydraulic implications. Note that not all branches of *Agathis australis* have an abscission zone, as adult foliage branches have a rough breakage zone and the xylem is not reduced (Wilson *et al.*, 1998). Unlike *W. nobilis* the *Agathis australis* branch base is much swollen with proliferation of parenchyma in the cortex. Similar to *W. nobilis* the xylem bends sharply down where it joins the main stem xylem, and there is a decrease in cross-sectional area of vascular tissue where the branch joins the trunk. Licitis-Lindbergs (1956) emphasized the presence of 'soft' xylem in the branch base due the parenchyma rays and that a partial segregation of the xylem weakens its strength. These features, in a much more pronounced form, are also found in *W. nobilis* (Figs 3A, C, 4, 6B, 9 and 10A).

Interestingly, *W. nobilis* branches are upright when first formed, but while they remain relatively straight or adopt a ' ∼ ' shape they begin to bend down, often bending through $>120°$ during their life (Fig. 1). The downward bending is probably related to the progressive increase in weight of a branch. Interestingly, given the small area of weak wood in the abscission zone, the branch base maintains a relatively constant angle with the stem. Most of the bending occurs further along the branch. Branches are often 80–120 cm long and will, at some stage, support a male or female strobilus at the tip. Even at the apparent base the oldest and longest branches are quite slender, with $<0.3$ cm$^2$ of xylem TS area (Fig. 8).

## CONCLUSIONS

*Wollemia nobilis* has the unique architectural features of unbranched branches and having all branches cleanly abscised. *Wollemia nobilis* has a pronounced morphological constriction in the branch-base xylem (usually four to six times greater xylem cross-sectional area in the apparent branch base than in the constriction). The constriction wood appears to be weak due to a very high proportion of ray parenchyma. *Wollemia nobilis* has very low Huber values for a gymnosperm and thus supplies a relatively large leaf area through a relatively small xylem area. The tracheids in the constriction do not appear to have a particularly large average lumen diameter or unusual frequency distribution. Relative theoretical hydraulic conductivity calculations indicate that the constriction has a conductivity that is, on average, 3·7 times lower than the apparent branch base. Taken together, these observations and measurements indicate that *W. nobilis* has evolved a unique modification of the xylem that facilitates abscission of all primary branches but may, under certain environmental conditions, restrict water supply to the foliage.

Possible poor vascular integration at various points in the *W. nobilis* plant and the presence of stomatal plugs, combined with studies that show that plants can compensate for various modifications to hydraulic pathways, indicate the constriction may not be of significance in whole plant water relations. Hopefully the above morphological and anatomical observations of the *W. nobilis* branch base will be of assistance in designing and interpreting subsequent physiological studies.

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